1: J Cell Biochem 1997 Feb;64(2):258-72

Purification and substrate specificity of polydeoxyribonucleotide kinases isolated from calf thymus and rat liver.

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Damage to DNA can result in strand breaks with 5'-hydroxyl and 3'-phosphate termini. Before DNA polymerases and ligases can rejoin the broken strands, such termini have to be restored to 5'-phosphate and 3'-hydroxyl groups. Polydeoxynucleotide kinase is an enzyme that may fulfil this function. We have purified the kinases from calf thymus and rat liver to near homogeneity. Based on SDS-polyacrylamide gel electrophoresis and activity gels, the enzymes from both sources are approximately 60-kDa polypeptides. Both enzymes have an acidic pH optimum (5.5-6.0) for kinase activity, and similar pI values (8.5-8.6), and a specificity for DNA. The calf thymus kinase possesses a 3'-phosphatase activity, as has previously been shown for the rat liver enzyme. The minimum size of oligonucleotide that can be labelled is 7-8 nucleotides in length, but the optimal size appears to be > 18 nucleotides. Comparison of phosphorylation of oligo(dA)24 and oligo(dT)24 with oligonucleotides containing a varied nucleotide sequence indicated that the homopolymers are poorer substrates. Unlike the bacteriophage T4 polynucleotide kinase, the mammalian kinases exhibit no preference for 5'-overhanging termini when acting at DNA termini produced by restriction enzymes. With double-stranded oligonucleotide complexes designed to mode single-strand gaps and nicks, the mammalian kinases preferentially phosphorylate the 5'-terminus associated with the gap or nick, in keeping with the idea that the kinases are involved in the repair of DNA single-strand breaks.

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5'phosphorylation of DNA in mammalian cells: identification of a polymin P-precipitable polynucleotide kinase.

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Proteins that catalyze 5' phosphorylation of an oligodeoxyribonucleotide substrate can be fractionated by polymin P treatment of whole cell extracts of calf thymus glands. Anion exchange chromatography on Q-Sepharose revealed three separable peaks of activity in the polymin P supernatant fraction, and one peak of activity in the Polymin P pellet fraction. The latter activity, Polymin P-precipitable polynucleotide kinase (PP-PNK), was further purified with a 1,500-fold increase of specific activity compared to the crude Polymin P pellet fraction. Oligonucleotides, a dephosphorylated 2.9-kb EcoRI fragment, and poly (A) were phosphorylated by the enzyme preparation, but thymidine 3' monophosphate was not a substrate. PP-PNK preparations exhibited an apparent KM of 52 microM for ATP and 8 microM for oligo dT25. The enzyme preparation displayed no detectable 3' phosphatase or cyclic 2',3' phosphohydrolase activities. The sedimentation coefficient of the PP-PNK activity was 3.8S as determined by sucrose density gradient analysis; the Stokes radius was 45 A, leading to an estimated molecular mass of 72 kDa. The enzyme had a pH optimum in the neutral to alkaline range in several buffer systems and is distinct from the DNA kinase with an acidic pH optimum previously described in calf thymus.

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